Published in final edited form as:

Arch Oral Biol. 2016 November ; 71: 1–9. doi:10.1016/j.archoralbio.2016.06.019.

Tongue implant for assistive technologies: Test of migration, tissue reactivity and impact on tongue function

Sylive Mimche^a, Dukju Ahn^b, Mehdi Kiani^b, Hassan Elahi^b, Kyle Murray^b, Kirk Easley^c, Alan Sokoloff^a, and Maysam Ghovanloo^{b,*}

^aDepartment of Physiology, Emory University School of Medicine, 615 Michael St., Atlanta, GA 30322, United States

^bSchool of Electrical and Computer Engineering, Georgia Institute of Technology, 85 Fifth Street NW, Atlanta, GA 30308, United States

^cBiostatistics and Bioinformatics, Rollins School of Public Health, 1518 Clifton Road NE, Atlanta, GA 30322, United States

Abstract

Objective—The Tongue Drive System (TDS) is a new wearable assistive technology (AT), developed to translate voluntary tongue movements to user-defined computer commands by tracking the position of a titanium-encased magnetic tracer (Ti-Mag) implanted into the tongue. TDS application, however, is constrained by limited information on biological consequence and safety of device implantation into the tongue body. Here we implant a stainless-steel pellet in the rat tongue and assay pellet migration, tongue lick function, and tongue histology to test the safety and biocompatibility of unanchored tongue implants.

Design—Water consumption, weight and lick behavior were measured before and for >24 days after implantation of a stainless-steel spherical pellet (0.5 mm) into the anterior tongue body of twelve adult male rats. X-rays were obtained weekly to assess pellet migration. Pellet location and tissue reaction to implantation were determined by post-mortem dissection and histology of the anterior tongue.

Results—By dissection pellets were distributed across the transverse plane of the tongue. Measures of water consumption, weight, and lick behavior were unchanged by implantation except for a decrease in consumption immediately post-implantation in some animals. By X-ray, there was no migration of the implant, a finding supported by pellet encapsulation demonstrated histologically. Measures of lick behavior were minimally impacted by implantation.

Conclusion—A smooth spherical stainless-steel implant in the anterior tongue of the rat does not migrate, is encapsulated and does not substantially impact lick behavior. These findings support the implantation of small tracers in the anterior tongue in humans for operating wearable assistive technologies.

*Corresponding author at: School of Electrical and Computer Engineering, Georgia Institute of Technology, 85 Fifth Street NW, Room TSRB-419, Atlanta, GA 30308-1030, United States. mgh@gatech.edu (M. Ghovanloo).

Keywords

Tongue; Tongue Drive System; Magnet; Implant; Spinal cord injury

1. Introduction

Some 12,000 individuals in the United States suffer spinal cord injury (SCI) each year; of these, individuals with incomplete (39.5%) and complete (16.9%) tetraplegia have limited options for and high costs of assistive devices (National Spinal Cord Injury Statistical Center, Spinal Cord Injury Facts and Figures, 2011). Despite advances in neuroprostheses and brain-computer interfaces, individuals with high-level SCI (i.e. C4 and above) and other neurological diseases which lead to tetraplegia, such as amyotrophic lateral sclerosis and brainstem stroke, have limited options for control of their environments. Currently such control relies upon interaction via a voluntary motor system unaffected or less affected by the SCI, typically either muscles of the face or neck. However, these systems provide limited signal diversity and bandwidth for computer command and cannot be used over extended periods.

Tongue motor innervation by cranial nerve XII is spared, even in individuals with high-level SCI. With its many degrees of freedom, facility for production of precise voluntary movements and non-fatiguing muscle profile, the tongue would thus appear to be a natural interface between cognition and computer for these individuals. Heretofore, the unique inherent abilities of the tongue as an interface has not been fully developed due to limitations in translating voluntary tongue movements in a precise, reproducible, unobtrusive, practical and safe manner. These limitations arise from the inaccessibility of the tongue inside the oral cavity and the requirement that interfaces do not impair preservative functions of the tongue in respiration, oral transport and swallowing, as well as speech.

The Tongue Drive System (TDS) was specifically designed with these considerations in mind. A key innovation of the TDS is the wireless and contactless translation of tongue movements via a small titanium-encased permanent-type magnet (Ti-Mag) affixed to the anterior tongue (Huo & Ghovanloo, 2010; Kim et al., 2012; Laumann et al., 2015). The TDS consists of an array of 3-axial magnetic field sensors, mounted near the user's cheeks on a headset to track the position of the tiny magnetic tracer, the size of a lentil (4.8 mm length, 1.5 mm thickness). Any tongue movement results in changes in the magnetic field inside and around the user's mouth. The electronics on the headset wirelessly transmit the measured magnetic field variations from all sensors to a nearby PC or smartphone, which runs a sensor signal processing (SSP) algorithm that attenuates the earth's magnetic field (EMF) components from the incoming signals, followed by a magnetic signal classification method that can currently indicate 7 distinct positions of the magnetic tracer within the 3-D oral space in real-time. These tongue gestures are then translated to specific user-defined commands, and sent to target devices, e.g., a wheelchair (Huo & Ghovanloo, 2008; Kim et al., 2012).

The effectiveness of the TDS has been demonstrated in both SCI and healthy individuals in pilot studies with magnets attached by temporary adhesion to dorsal tongue epithelium or by

tongue piercing (Huo & Ghovanloo, 2010; Kim et al., 2013). However, these approaches are impractical for long term use due to lingual and dental complications, the requirement of persistent hygienic maintenance and resistance by older patients to tongue piercing. An optimal solution is to implant the Ti-Mag tracer in the tongue body obviating the need for implant maintenance (as with tongue piercing) and minimizing the risk of swallowing the magnetic tracer or damaging teeth and gums.

Despite these advantages, features unique to tongue biology might negatively impact the safety and reactivity of implantation of a free-standing device in the anterior tongue. Complex changes in tongue stress-strain patterns during oromotor behaviors (e.g., Felton et al., 2008) as well as routine tonic and forceful tongue muscle activation in preservative behaviors (i.e., respiration, swallowing) may impact the formation of a sequestering implant capsule. Tongue muscles are highly capillarized (Granberg, Lindell, Eriksson, Pedrosa-Domellof, & Stal, 2010) and the presence of large ventral lingual arteries and branches raises the possibility of embolization if a small device is not appropriately integrated into surrounding tissue.

To assess safety and reactivity of implantation of a small, unanchored device in the anterior tongue we assayed implant migration, tongue lick function and tongue histology following injection of a 0.5 mm stainless-steel pellet into the rat tongue. Our findings reveal only minimal and temporary impact of implantation on tongue biology.

2. Materials and methods

2.1. Husbandry and measurement of rat licking

All procedures were conducted in accordance with Georgia Institute of Technology Institutional Animal Care and Use Committee. Animals were housed singly to enable individual collection of water consumption and lick data. Water (500 cc bottle) and food was offered *ad libitum*. Animals were weighed at least weekly from at least 4 days before to at least 24 days after implantation. Water consumption (weight change of water in bottle in grams) was determined on most days but sometimes was expressed as the average of 2–4 days of consumption. Actual or average daily water consumption was related to measures of lick behavior assessed during the period of consumption. We sought to measure licking under normal conditions to create a baseline and assess the possible impact of implantation on drinking behavior; therefore we collected lick data continuously and did not constrain access to the nipple. Lick measures were collected for at least 6 days prior to implant using capacitive and resistive custom systems designed at Georgia Tech. Briefly, the capacitive lick meter was designed to measure changes in capacitance between the stainless-steel nipple of the water bottle and instrument ground. The only electrode of the capacitive lick meter was wrapped around the neck of the stainless-steel nipple without changing its shape or characteristics. When the rat tongue touched the nipple, the animal's body changed the capacitance between nipple and ground, which was detected using an AT42QT1011 touch sensor integrated circuit (Atmel, San Jose, California; see Fig. 1). The resistive lick meter required two electrodes and monitored changes in the impedance between these two electrodes during licking. One electrode was attached to the stainless-steel nipple and the other, which served as the ground electrode, was a metal grid spread over the floor of

homage. When the animal tongue touched the nipple, a low resistive path was created between the two electrodes changing the impedance detected by the circuit. The outputs of both types of lick meters were sampled by a MSP430 microcontroller (Texas Instruments) and delivered to a personal computer via Universal Serial Bus (USB) every 11.2 ms (~90 Hz).

2.2. Implant procedure

Twelve adult male Sprague-Dawley rats (291–412 g, assigned identification GT1-G12) were induced with isoflurane in a chamber, masked, maintained with isoflurane (2.25–5%) and placed supine on a heating pad (~37 °C). The mouth was opened, the tongue was gently held at the tip with forceps and wiped with alcohol. A sterilized stainless-steel spherical pellet (0.5 mm diameter, Salem Specialty Ball, Connecticut) was injected through the anterior tongue dorsum into the anterior tongue body by sterile injector assembly (syringe, 20-gauge needle and wire plunger). Occasional minimal bleeding in response to injection was resolved by gentle pressure with a sterile cotton-tipped applicator. Following implantation stainless-steel pellets were glued to the tongue epithelium (cyanoacrylate glue) at the tongue tip and frenulum to facilitate localization of the implant by X-ray. Under continued anesthesia, lateral and ventral-dorsal X-rays were acquired using a Faxitron MX-20 Digital X-Ray set at 27 kV and 35 kV with 15 s exposure time to view both soft tissue and the implant with sufficient contrast (Faxitron Bioptics, LLC, Tucson, Arizona).

2.3. Schedule of X-rays to track pellet migration

Following implantation X-rays were taken weekly to evaluate pellet migration: rats were anesthetized with isoflurane, reference pellets were glued to the tongue tip and frenulum and X-rays acquired as described above. In the final X-ray session additional pellets were glued to the tongue surface to aid in post-mortem localization of the implant. In total each rat underwent five X-rays sessions.

2.4. Tissue harvest

Following the final X-ray (days 24–28 post-implant), rats were deeply anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg), the chest cavity was opened, and animals were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde at room temperature. Tongues were removed, post-fixed in 4% paraformaldehyde for up to 2 weeks and transferred to 20% sucrose in 0.1 M phosphate buffer. By reference to external pellets on the tongue and corresponding X-rays taken before sacrifice, tongues were cut in the transverse plane into blocks of ~5–8 mm with one cut targeted immediately anterior to the implant. The block with the implant was photographed *en face* with the implant *in situ*, and the implant was removed with sharp forceps either at room temperature or while the tongue block was cooled by crushed dry ice. In one rat, the tongue block was mounted on a tongue depressor with OCT Tissue TEK (Sakura, Finetek), frozen, stored at –80° C and cut in 12-µm sections by cryostat (–23°; Leica). All other tongues were prepared for conventional paraffin embedding: the tissue block was trimmed to include the pellet cavity and embedded in paraffin for sectioning (9 µm-thickness) in either transverse or parasagittal planes.

2.5. Histochemical procedures

Slides were reacted for conventional hematoxylin and eosin (HE, Hematoxylin Solution, Harris Modified, Sigma) and picrosirius red (PS, NovaUltra Sirius Red Stain Kit) histochemistry, dehydrated, and coverslipped in permount. Additional slides were prepared for immunohistochemistry by epitope exposure to unmask antigenic sites. However, we were unable to label tongue tissue with antibodies to developmental myosin heavy chain and to molecular markers of inflammation and wound healing (e.g., anti-CD68, anti-F4/80, anti-smooth muscle actin).

2.6. X-ray analysis of implant migration

The tongue is a soft tissue structure and tongue body shape is influenced by body orientation and anesthetic state. Differences in head alignment, external pellet placement and tongue posture between X-ray sessions precluded precise alignment of anatomical and external markers across sessions. We, therefore, sought to determine whether there was systematic migration of the implant over the course of the experiment. Lateral X-rays were imported into Neurolucida Software (MBF Bioscience, Williston, Vermont), scaled to the 0.5 mm diameter implant, and digital markers were placed on the implant and on external tongue tip and frenulum pellets. When identifiable, the contour of the tongue dorsum was traced. Distance of the anterior tongue tip to the implant (line AI) and from the implant to the frenulum (line IP) and the location of the implant relative to the contour (distance to contour; distance along contour from tip) was calculated by the software (see Fig. 2). Data were exported into Excel and implant migration was evaluated by determining implant location relative to external markers and the tongue contour for each X-ray session. The angle formed by lines AI and IP and the distance from tongue tip to frenulum markers (line AP) was calculated (Fig. 2). Disparity in location of tongue tip and frenulum markers varied between X-ray sessions, likely reflecting inter-session variability in marker placement and in tongue posture. Therefore we restricted analysis to X-ray sessions in which the AP distance was within 20% of the initial AP distance. Distance measures were graphed in Excel and in Statistica software (Dell, Round Rock, Texas).

2.7. Implant localization and histology

Location of the implant in the transverse plane was determined from gross dissection and from sections reacted for HE and PS. Capsule thickness was determined from HE sections in Neurolucida software: thickness was measured at 8–12 ~equidistant locations around the capsule perimeter on at least 2 different tissue sections for each animal. Capsule width measurements for each animal were averaged. Damage to capsule tissue during implant removal precluded measurement of the implant capsule in 3 of 12 rats. To eliminate variability due to differences in tissue processing, GT6, which was not embedded in paraffin, was not included in analysis of capsule thickness.

2.8. Analysis of lick data

Licking behavior and water consumption were measured daily to determine (1) the time spent by each animal at the nipple (i.e., total lick time, TLT) and (2) temporal features of lick cycles in discriminated licks (tongue on-nipple time, TON, and tongue off-nipple time,

TOF). Because access to the nipple was not constrained, some licks occurred without disruption of rat-nipple contact. Therefore, we defined a lick bout as any period of rat-nipple contact up to five minutes followed by at least five seconds of no rat-nipple contact. Lick-bouts were summed over the period of data collection to determine total lick time (TLT) and water consumed during the period of data collection was divided by TLT to determine lick efficiency (LE, i.e., water released/time at nipple). Individual licks were defined by a sequence of TON-TOF-TON for which TON and TOF were 30–300 ms in duration. TON and TOF for cycles meeting these criteria were analyzed by day to evaluate changes in lick microstructure following implantation.

Outcomes included tongue on time (TON), tongue off time (TOF) and total lick cycle time (TLT). These outcomes were measured before and after implantation for each of 11 animals. The analysis of repeated measures data is needed that properly accounts for the correlation between multiple observations from the same animal. The method of least squares was used to fit a general linear model that provided the estimated outcome mean and 95% confidence interval for each day before and after implantation. Paired *t*-tests were used to compare the differences between the model-based means (least-squares means) before and after implantation. Specific statistical tests were done within the framework of the general linear model. Rates of increase of LE (log) were obtained using a mixed-effects model specifying that LE follows a linear regression over time of study, with a random intercept for each animal. The mean slope or the average rate of increase of LE over approximately 30 days was estimated and compared to zero using a *t*-test within the framework of the mixed-effects model.

3. Results

3.1. Animal biology

Measures of water consumption for all animals were unchanged by implantation with the exception of a decrease in consumption on the days immediately following implantation in some animals (Fig. 3). Animal weight was not affected by implantation (average $\pm$ standard deviation of weight before implant 359.1 ± 32.3 g and at end of experiment 392.6 ± 47.9 g).

Lick efficiency (LE) did not decline after implantation and there was a slight but statistically significant linear increase in LE in pooled data (Fig. 4A; $P=0.038$ testing slope=0).

Individual comparisons of LE revealed a decrease in one and increase in three subjects post-implant. Duration of TLT and OT increased whereas duration of TO decreased following implantation (Table 1 and Fig. 4B–D).

3.2. Implant migration

Nine animals met criteria for X-ray analysis (Fig. 5). Measures of implant-to-tongue contour distance and implant-to-tongue tip distance (described along the path of the tongue contour) differed between X-ray sessions. We attribute these non-systematic differences to variable posture of the tongue and variable placement of reference pellets by X-ray session.

3.3. Implant location and capsule histology

Gross dissection and HE and PS histology demonstrated encapsulation of the implant (Fig. 6). Implant locations were distributed across the transverse plane of the tongue body (Fig. 7). Obvious damage to the capsule during implant removal precluded capsule analysis in three subjects. Two capsule morphologies were present. In five subjects implant capsules were comprised of a circular band of dense, organized collagen populated by many fibroblasts (Fig. 8A–I). In some of these subjects a thin outer connective layer could be distinguished (Fig. 8, Arrows). In four subjects the implant cavity was demarcated by a fine ring of collagen (Fig. 8J–L). Average thickness of all capsules was $17.8 \pm 10.2 \mu\text{m}$ (Table 2). By HE markers of chronic inflammation were not observed adjacent to the implant capsule: Lymphocytes and macrophages were few and foreign body giant cells were not present. Tongue muscle adjacent to the capsule appeared normal and few fibers exhibited central nuclei indicative of degeneration/regeneration of muscle fibers.

4. Discussion

4.1. Summary

Implantation of a spherical stainless-steel implant into the anterior tongue of the rat does not affect animal weight, water consumption, or lick behavior. Implant encapsulation and the absence of implant migration indicate a rapid integration of the implant into tongue tissue minimizing the risk of embolization into large ventral lingual arteries. These findings suggest the safety and biocompatibility of implantation of small biomedical devices into the anterior tongue in humans.

4.2. Considerations of tongue biology on implantation

Kinematic, anatomic and neural features unique to tongue biology may impact the safety of an unanchored device in the anterior tongue. Complex changes in tongue stress-strain patterns (e.g., Felton et al., 2008) as well as routine tonic and forceful tongue muscle activation in preservative behaviors (i.e., respiration, swallowing) might impact the formation of a sequestering implant capsule resulting in persistent local inflammation or implant migration. Tongue muscles are highly capillarized (Granberg et al., 2010) and the presence of large ventral lingual arteries raises the possibility of embolization if a small device is not integrated into surrounding tissue. Additionally, the high bacterial load of the oral cavity may promote introduction of foreign bodies during implantation with bias to an inflammatory response. Following tongue piercing in the dog for example, foreign-body-like giant cells persist for 3 months, a putative reaction to hair and food particles introduced during piercing (Lopez Jornet et al., 2004).

Despite these concerns, prior studies indicate that implantation of unanchored devices into the tongue is well-tolerated. In dogs a polymer-coated magnet of $\sim 2.5 \times 15 \times 25 \text{ mm}^3$ inserted into the posterior tongue base was encapsulated, did not migrate and did not cause systemic acute or chronic inflammation (Nelson, Boucher, & Stevens, 2005). Magnet insertion was associated with 6.4% weight loss with recovery by two weeks. In the infant mini-pig insertion of up to six metal rods $\sim 9 \text{ mm}$ diameter $\times$ 1–3 mm length into the tongue did not interfere with normal feeding (German, Crompton, Levitch, & Thexton, 1992). The

present study extends prior findings by demonstrating encapsulation without local inflammation, no migration, maintenance of animal weight and minimal impact on lick behavior following implantation of a smooth spherical stainless-steel pellet into the anterior tongue.

4.3. Tongue tissue response to implant

Factors specific to implant design, material and tissue determine the biological response to implantation of a metallic device (for review see Anderson, Rodriguez, & Chang, 2008; Woodward and Salthouse, 1986). Following implantation of a 6 mm × 1.8 mm stainless-steel or titanium rod into paraspinal muscles in the rat Ryhanen et al. (1998) reported a ~55 µm-thick capsule with a distinct inflammatory layer and occasional foreign body-giant cells. In contrast, limited inflammation and a fine capsule (10 µm thickness) were present 10 days following insertion of a 5 mm × 0.5 mm stainless-steel or titanium wire into the mouse tibialis anterior muscle (McGeachie, Smith, Roberts, & Grounds, 1992). McGeachie et al. hypothesized that rapid maturation to a thin capsule was related to the mobility of the tibialis anterior muscle. By this hypothesis, routine tongue muscle activation and movement during respiration, licking, and feeding might promote maturation of an implant capsule. Indeed, similar to McGeachie et al. (1992), at ~4 weeks post-implantation we observed limited inflammation, no foreign body giant cells and, in some subjects, a fine fibrous capsule layer 10 µm thick. In other subjects we observed a robust capsule of multiple layers of fibroblasts and collagen, similar in morphology to titanium encapsulation at 4 weeks following implant into rat back musculature (Ryhanen et al., 1998). In our study, capsule morphology was not related to implant location. Individual variation in capsule thickness was reported by Ryhanen et al. (1998) and may explain the range of capsule morphologies in our study.

4.4. Consequences of implantation for licking

To our knowledge previous studies have not quantitatively assessed the impact of tongue implant on lick behavior. Lick cycle duration in our study was similar to prior reports in rats (e.g., 4–7.5 Hz, Weijnen, 1998). There was a significant increase in total lick cycle duration (TLT) and tongue on time (TON) and decrease in tongue off time (TOF) following implantation. These changes in lick measures were slight however (3.03, 4.85 and –1.83 ms respectively) and may not have biological importance. Lick efficiency (LE) also increased slightly following implantation. In concert these outcomes indicate that lick behavior was not substantially changed by implantation of the pellets.

4.5. Limitations of study

We recognize several limitations to the current study. First, we cannot rule out movement of the pellet in the first days following implantation. Sequential X-rays, however, indicate that movement, if present, was minimal. Second, post-mortem removal of the implant, necessary for tissue processing, may have damaged the capsule biasing morphological analysis. Counter to this possibility is the consistency of type of capsule morphology throughout each tissue section. Finally, although LE and tongue-nipple contact time (TON) increased slightly after implantation, we did not include a control study of similar age *sans* implant; thus we

cannot rule out the contribution of increasing age and animal size to changes in lick measures.

5. Conclusion

Our study demonstrates that a smooth spherical implant in the anterior tongue of the rat does not migrate, is naturally encapsulated, and does not impact lick behavior. These findings support the implantation of devices in the anterior tongue in humans for assistive technologies or other medical applications.

Acknowledgements

The authors thank Dr. Laura O'Farrell and Ms Kimberley Benjamin for assistance with animal care, Dr. Marla Gearing, Ms Deborah Cooper and Mr Robert Baul at the Emory Neuroscience NINDS Core Facilities Neuropathology and Histochemistry Core (Grant number iP30 NS05507) for paraffin processing and histology assistance. Supported by NIH Grant R21EB016662 to AJS and MG.

References

- Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Seminars in Immunology*. 2008; 20(2):86–100. <http://dx.doi.org/10.1016/j.smim.2007.11.004> S1044-5323(07)00096-6[pil]. [PubMed: 18162407]
- Felton SM, Gaige TA, Benner T, Wang R, Reese TG, Wedeen VJ, et al. Associating the mesoscale fiber organization of the tongue with local strain rate during swallowing. *Journal of Biomechanics*. 2008; 41(8):1782–1789. <http://dx.doi.org/10.1016/j.jbiomech.2008.01.030> S0021-9290(08)00025-0[pil]. [PubMed: 18456271]
- German RZ, Crompton AW, Levitch LC, Thexton AJ. The mechanism of suckling in two species of infant mammal: miniature pigs and long-tailed macaques. *Journal of Experimental Zoology*. 1992; 261(3):322–330. <http://dx.doi.org/10.1002/jez.1402610311>. [PubMed: 1629663]
- Granberg I, Lindell B, Eriksson PO, Pedrosa-Domellof F, Stal P. Capillary supply in relation to myosin heavy chain fibre composition of human intrinsic tongue muscles. *Cells Tissues Organs*. 2010; 192(5):303–313. <http://dx.doi.org/10.1159/000318645> 000318645[pil]. [PubMed: 20616532]
- Huo X, Ghovanloo M. Evaluation of a wireless wearable tongue-computer interface by individuals with high-level spinal cord injuries. *Journal of Neural Engineering*. 2010; 7(2):26008. <http://dx.doi.org/10.1088/1741-2560/7/2/026008> S1741-2560(10)28441-0[pil]. [PubMed: 20332552]
- Kim J, Huo X, Minocha J, Holbrook J, Laumann A, Ghovanloo M. Evaluation of a smartphone platform as a wireless interface between tongue drive system and electric-powered wheelchairs. *IEEE Transactions on Biomedical Engineering*. 2012; 59(6):1787–1796. <http://dx.doi.org/10.1109/TBME.2012.2194713>. [PubMed: 22531737]
- Laumann A, Holbrook J, Minocha J, Rowles D, Nardone B, West D, et al. Safety and efficacy of medically performed tongue piercing in people with tetraplegia for use with tongue-operated assistive technology. *Top Spinal Cord Inj Rehabil*. 2015; 21(1):61–76. <http://dx.doi.org/10.1310/sci2101-61>. [PubMed: 25762861]
- Lopez Jornet P, Vicente Ortega V, Yanez Gascon J, Cozar Hidalgo A, Perez Lajarin L, Garcia Ballesta C, et al. Clinicopathological characteristics of tongue piercing: an experimental study. *Journal of Oral Pathology and Medicine*. 2004; 33(6):340–345. <http://dx.doi.org/10.1111/j.1600-0714.2004.00219.x> JOP219[pil]. [PubMed: 15200482]
- McGeachie J, Smith E, Roberts P, Grounds M. Reaction of skeletal muscle to small implants of titanium or stainless steel: a quantitative histological and autoradiographic study. *Biomaterials*. 1992; 13(8):562–568. [PubMed: 1633231]
- National Spinal Cord Injury Statistical Center, Spinal Cord Injury Facts and Figures. *Journal of Spinal Cord Medicine*. 2011; 34(6):620–621. [PubMed: 22330120]

- Nelson LM, Boucher RP, Stevens SS. Magnetic airway implants for the treatment of obstructive sleep apnea syndrome. *Otolaryngology—Head and Neck Surgery*. 2005; 133(6):954–960. <http://dx.doi.org/10.1016Zj.otohns.2005.08.022> S0194-5998(05)01765-1[pii]. [PubMed: 16360520]
- Ryhanen J, Kallioinen M, Tuukkanen J, Junila J, Niemela E, Sandvik P, et al. In vivo biocompatibility evaluation of nickel-titanium shape memory metal alloy: muscle and perineural tissue responses and capsule membrane thickness. *Journal of Biomedical Materials Research*. 1998; 41(3):481–488. [http://dx.doi.org/10.1002/\(SICI\)1097-4636\(19980905\)41:3<481:AID-JBM19>3.0.CCI;2-L\[pii\]](http://dx.doi.org/10.1002/(SICI)1097-4636(19980905)41:3<481:AID-JBM19>3.0.CCI;2-L[pii]). [PubMed: 9659619]
- Weijnen JA. Licking behavior in the rat: measurement and situational control of licking frequency. *Neuroscience and Biobehavioral Reviews*. 1998; 22(6):751–760. S0149763498000037[pii]. [PubMed: 9809310]
- Woodward, SC.; Salthouse, TN. *Handbook of biomaterials evaluation: scientific, technical and clinical testing of implant materials*. Macmillan; New York: 1986. The tissue response to implants and its evaluation by light microscopy; p. 364-377.

Fig. 1.

The schematic of the lick meter interface, the main component of which is a single-key QTouch® touch sensor chip (AT42QT1001, Atmel, San Jose, CA). This chip comes in a small SOT23 package and its touch sensitivity can be adjusted with an external capacitor (1 μ F in this case).

Fig. 2.

X-ray analysis of implant migration. (A) Lateral X-ray of GUI with tongue contour (dotted line), implant and markers at tongue tip and lingual frenulum. (B) Distance from implant to contour (short, straight, yellow line) and from tongue tip to implant along contour (long, curved, red line) was determined on X-rays for which the distance from tip marker to frenulum marker (line A-P was) was within 20% of the value at implantation (see Methods for further explanation). Calibration bar = 1.0 mm. (For interpretation of the references to colours in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Measures of animal weight (circles; grams) and water consumption (squares; grams) by day aligned to day of implantation (dotted line, day 0). Implantation did not negatively impact weight or water consumption with the exception of a decrease in consumption immediately following implantation in some animals (Fig. 3).

Fig. 4.

Measures of lick behavior were minimally affected by implantation. (A) The relation between amount of water consumed and time spent at the nipple (lick efficiency, LE) is shown for each subject. LE did not decline after implantation and there was a slight but significant linear increase in LE in pooled data during the experiment ($P = 0.038$). (B–D) Measures of lick microstructure before and after implantation in eleven animals. Following implantation lick cycle time increased (B, <0.001), tongue-on time increased (C, <0.001) and tongue off-time decreased (D, <0.001). The horizontal line is the mean at day zero. The 95% confidence intervals are tight around the mean.

Fig. 5.

Distance from the implant to tongue dorsum contour (circles) and from the implant to tongue tip (described along the path of the contour, squares) determined in weekly X-ray sessions. Only sessions that met inclusion criteria are shown. We attribute differences in distance measures between X-ray sessions to variable posture of tongue and variable placement of reference pellets by X-ray session.

Fig. 6. Localization of implant in transverse plane of the tongue body by dissection and histology (subject GT7). (A) Implant *in situ* prior to extraction. (B) Implant cavity after extraction. (C) HE stain of paraffin-embedded tissue showing implant capsule (arrows). Arrow head marks the median septum (midline) of the tongue body. Box in B indicates approximate location of photomicrograph in C. Calibration bars = 0.5 mm.

Fig. 7.
Location of implants in the transverse plane of the tongue body. For ease of illustration all implants are superimposed on a single side of the tongue.

Fig. 8.

Histology of the implant capsule demonstrated by Picrosirius Red (B, H, K) and Hematoxylin-Eosin staining. Two primary capsule morphologies are observed. In one a dense capsule of collagen and fibroblasts surrounds the implant cavity (subject GT4, A–C; subject GT8, D–F; subject GT10, G–I). Occasionally a thin outer fibrous layer is distinguished (arrows in F and I). In the other a thin fibrous layer surrounds the implant cavity (subjects GT7, J,K and GT11, L). All calibration bars 250 μ m except F (125 μ m).

Table 1Measurements of lick microstructure before and after implantation.^a

	Mean $\pm$ SEM ^b before implantation (ms)	Mean $\pm$ SEM after implantation (ms)	Average change (ms)	95% confidence intervals	P Value ^c
Total Lick Cycle Time (TLT)	149.9 $\pm$ 0.17	152.9 $\pm$ 0.16	3.02	2.88, 3.17	<0.001
Tongue On Nipple Time (TON)	70.3 $\pm$ 0.12	75.2 $\pm$ 0.12	4.85	4.75, 4.96	<0.001
Tongue Off Nipple Time (TOF)	79.6 $\pm$ 0.15	77.8 $\pm$ 0.15	-1.83	-1.96, -1.70	<0.001

^aTotal sample size 74,424 measurements before implantation and 282,863 measurements after implantation.^bStandard Error of the Mean.^cP value is comparing pooled pre-implantation versus pooled post-implantation mean difference.

Table 2

Average capsule thickness by subject.

Subject ID	Mean $\pm$ SD (μm)
All experiments	17.8 $\pm$ 10.2
GT1	10.1 $\pm$ 3.3
GT3	22.5 $\pm$ 1.8
GT6	13.4 $\pm$ 5.9
GT7	29.5 $\pm$ 13.9
GT9	19.6 $\pm$ 7.6
GT10	9.1 $\pm$ 2.5
GT11	17.1 $\pm$ 7.0
GT12	12.9 $\pm$ 4.1